TRIPLICATION VS. DUPLICATION IN REGENERATED ARTHROPOD LIMBS: MULTIPLE STABLE STATES IN THE SHAPING OF CELL SHEETS

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Abstract. A model for the shaping of cell sheets offers an interpretation for alternative structures which regenerate after a grafting operation. The model treats an epithelium as a thin shell which is fluid-like; cells in a small multicellular element can exchange neighbors to relieve shear stress. This fluidity allows interfacial tensions between groups of cells which differ in their adhesive affinities. The shell element bends elastically; its stiffness is associated with the cytoskeleton in each cell and with intercellular junctions. This fluid elastic shell model is used to model regeneration of the epithelium covering an insect or crustacean. The model suggests that mechanisms of regeneration minimize an energy functional. Typically regeneration restores the normal local pattern of structures everywhere in the epithelium. However, regenerated structures containing a few abnormal local patterns may also represent energy minima.

Keywords. Biomechanics; catastrophe; metastable; optimization; regeneration; shell.

INTRODUCTION

Many parts of an animal develop from sheets of cells, or epithelia. Mathematical models can help us to understand how an epithelium changes curvature and thickness to generate a biological form. The present work explores a model to explain how an epithelium can develop more than one stable shape. The epithelium, the hypodermis, covers the surface of arthropods such as insects and crustaceans. The hypodermis is a sheet of cells one cell thick; it generates the external form.

Many arthropods can repair the hypodermis after it is damaged, regenerating missing structures. If a leg is cut off, a new leg regenerates to replace it. However, if a piece of leg is grafted to a foreign host site in the hypodermis, diverse abnormal structures may regenerate. For example, in many species, after the end of a right leg is grafted to the stump of a left leg, a triplicated tip regenerates (Fig. 1, trip). The operation elicits the formation of two supernumerary ends, each with host symmetry, from opposite sides of the junction between the host and the graft.

This triplicated tip illustrates a rule which is often valid in regeneration, the principle of continuity: Regeneration typically restores the normal local pattern of structures (French et al., 1976; Winfree, 1980; Lewis, 1981; Mittenthal, 1981). Occasionally, however, after this operation only one supernumerary end develops (Fig. 1, dup). A discontinuity of pattern remains at the site where the other supernumerary is expected.

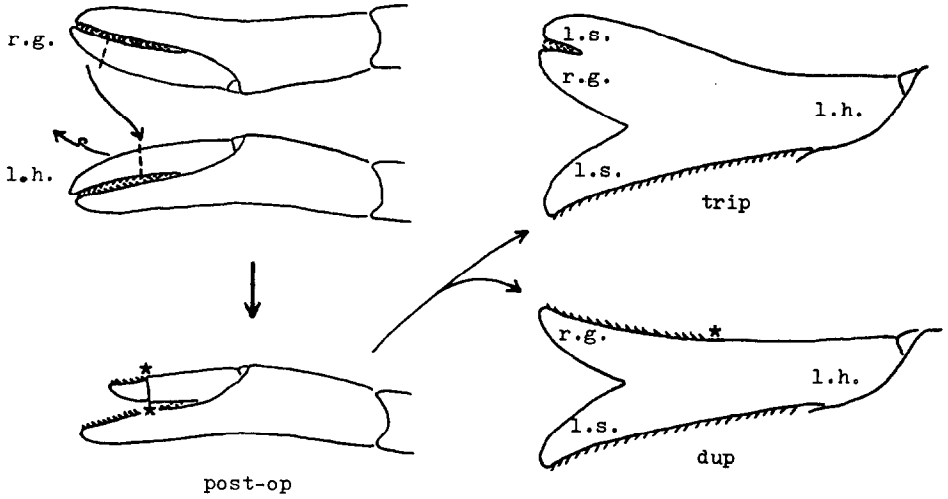

FIG. 1. Regeneration of a triplicated limb, illustrated for the terminal segment of a crayfish leg, the dactyl. A right dactyl (r.g.) is grafted into a left dactyl (l.h.). l.s., a supernumerary tip with host symmetry. limbs: post-op, post-operative; dup, duplicated; trip, triplicated. asterisk, site of maximum disparity.

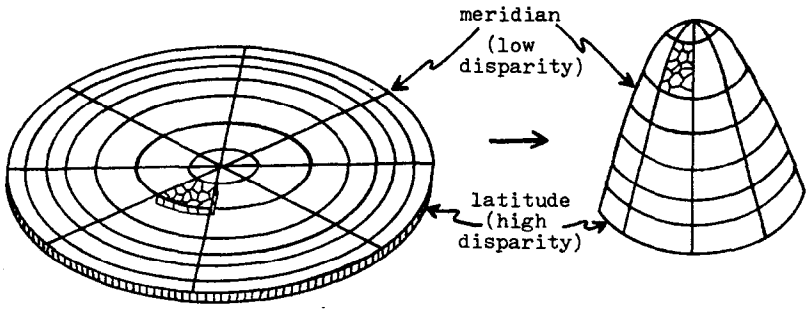

FIG. 2. Deformation of an epithelium containing a bull's eye pattern of cell types, from a planar disk to a blunt cone. Cells of one type occupy the intersection of a sector and an annulus.

MODEL

We would like to understand why the principle of continuity is usually valid, and why structures with discontinuities of pattern sometimes regenerate. A biomechanical model for the shaping of epithelia suggests interpretations for these phenomena. This model treats an epithelium as a thin shell (Mittenthal and Mazo, 1983; Mittenthal and McMeeking, 1986). A small multicellular element resists bending, or isotropic in-plane tension or compression, as does an elastic medium. Its stiffness comes from the filamentous skeleton in each cell and from junctions between cells. The elastic forces can be derived from an energy of bending strain; we ignore stretching here. The shell is also fluid-like, in that cells in the element can exchange neighbors to relieve shear stress. In a fluid sheet with cells of different types segregated into blocks, there will be interfacial tensions between the blocks; these can affect the shape of the sheet. The interfacial tensions can be derived from an adhesive energy which increases with the adhesive disparity between adjacent unlike cells.

This fluid elastic shell model suggests that the hypodermis of an arthropod tends toward the shape which minimizes an energy functional, the sum of the strain energy and the adhesive energy. Performing this minimization gives the equations of mechanical equilibrium for a thin shell -- for the balance of moments, balance of tangential forces, and balance of normal forces.

The balance between strain and adhesion can shape an epithelium. Fig. 2 shows a disk of epithelium which contains a bull's-eye pattern of cell types. All the cells of one type are located in one sector of an annulus. The adhesive disparity

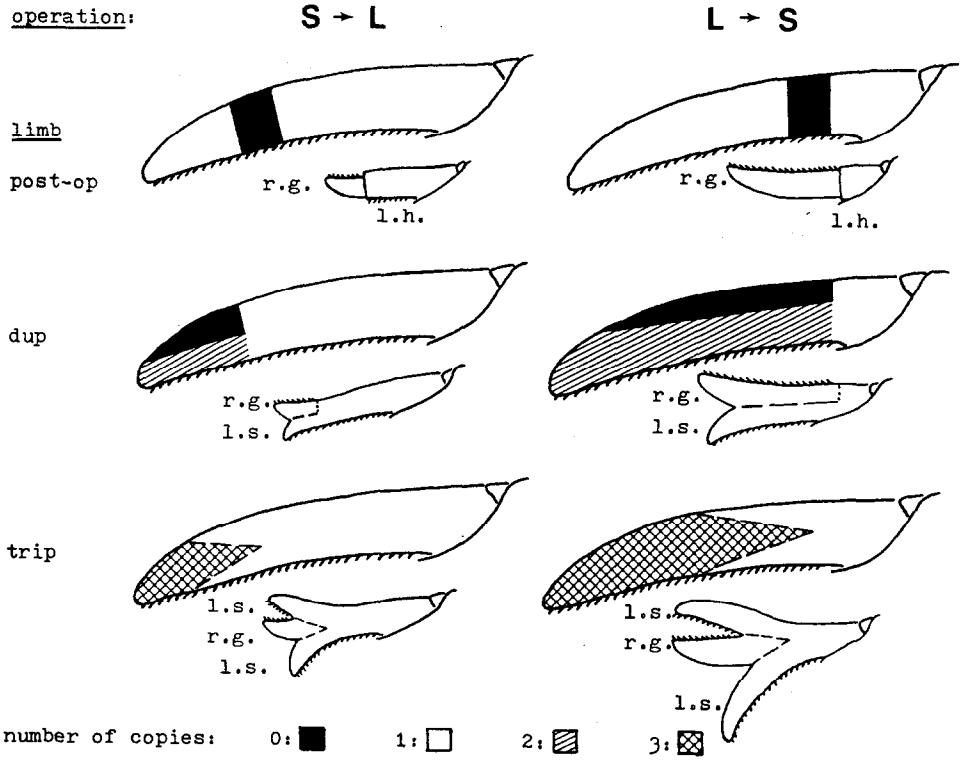

FIG. 3. Results of grafting operation; see Fig. 1. S-->L, short graft to long host stump; L-->S, long graft to short host stump. Small inset: appearance of limb; larger drawing: limb mapped onto a normal limb with the number of copies of each normal region indicated. Dashed curves are loci of mirror-symmetry; dotted curves are loci of pattern discontinuity remaining after regeneration.

between two adjacent annuli increases with the circumference of the annuli. The total adhesive disparity of the epithelium is reduced if the disk deforms into a blunt cone, because this deformation decreases the circumference of each annulus and the associated adhesive disparity. However, as the disk bends its strain energy increases. Thus, under the opposing influences of adhesion and strain, a blunt cone develops as a compromise shape.

Suppose this shape represents a normal limb (Mittenthal and Warga, 1983). If the grafting operation shown in Fig. 1 is performed on this model limb, what patterns of cell types will have minimum energy and will regenerate? To address this question we need to specify the energy associated with an arbitrary state -- an arbitrary arrangement of cell types, in an epithelium with an arbitrary shape. One can then plot surfaces of constant energy in a space of these states; these surfaces define an energy landscape. The state space has many dimensions, because the number of cell arrangements, and of shapes of the cell sheet, is large. However, features of the energy landscape are evident in a simplified two-dimensional state plane. The variables in this plane are

interfaces: the number of types of adhesive interfaces present. In Fig. 2, each border in the quilt pattern is a different type of interface. The number of these interfaces is the abscissa in the state plane.

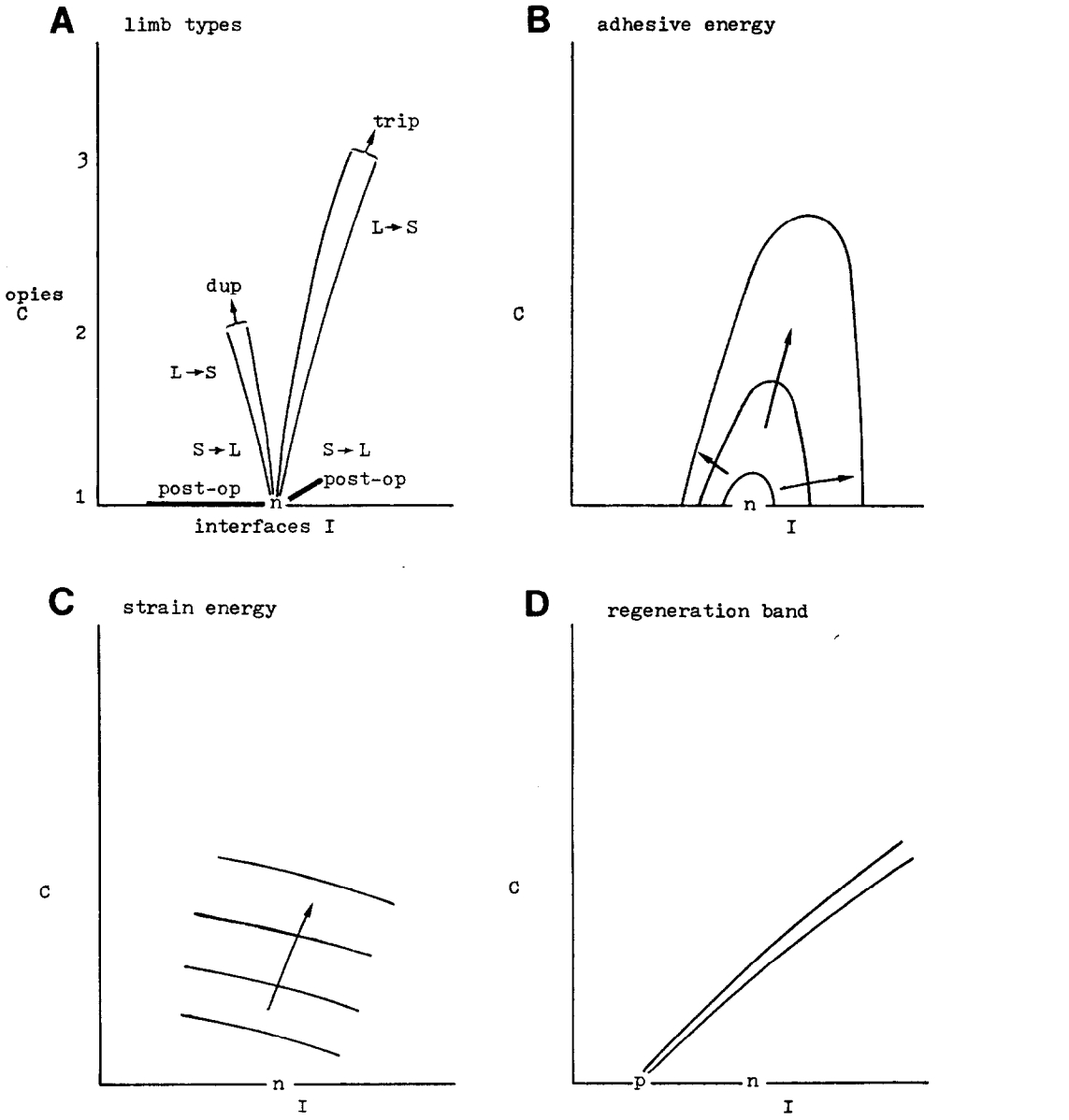

FIG. 4. The state plane. interfaces and copies are defined in text. n: normal limb. (A) Loci of limb types in Fig. 3. (The trip band should be vertical, but is displaced for clarity in subsequent drawings.) (B) Topographic map of landscape for adhesive energy, showing curves of constant energy. (C) Landscape for strain energy. Arrows in (B) and (C) indicate directions of increasing energy. (D) The regeneration band accessible from post-operative state p.

copies: the number of copies of each type of interface, averaged over all the types of interfaces in a limb. A normal limb has one copy of each interface, but some limbs resulting from the grafting operation in Fig. 1 have zero or multiple copies of some interfaces, as Fig. 3 shows.

A post-operative limb may have some cell types deleted in a band at the host-graft junction. A duplicated (dup) limb has some cell types deleted near the dorsal margin, so it lacks some normal interfaces. It has a few abnormal types of interfaces at the pattern discontinuity. A triplicated (trip) limb has three copies of some cell types. All the normal types of interfaces, and only those types, are present. In all these types of limbs the number of copies varies over the limb, taking on integer values at any point. However, the state variable copies is the average number of copies, averaged over the limb; this variable changes continuously with the extent of duplication or deletion.

Fig. 4A shows regions of the state plane corresponding to each of these limb types. A normal limb corresponds to a unique point, since it has a characteristic number of interfaces and one copy of each interface. A post-operative limb with cell types deleted has a subnormal number of interfaces with one copy of each. In the state plane a point representing such a post-operative limb lies on the interfaces axis, to the left of the normal limb. If the levels of cutting leave some cell types present in the graft and also in the host stump, interfaces and copies are greater than in a normal limb.

In the state plane of Fig. 4A, a dup limb has interfaces less than normal, and copies between 1 and 2. A dup with a longer region of duplication has more types of interfaces deleted, and a larger value of copies. A trip limb has copies between 1 and 3. If the region of triplication is longer, copies is larger, but interfaces remains at its normal value.

Heuristic arguments allow us to sketch the energy landscape on the state plane. We consider the landscapes of adhesive energy and strain energy separately, and then add them together. For the adhesive energy (Fig. 4B), if the number of types of interfaces is abnormally small or large, some abnormal interfaces must be present. Suppose that abnormal interfaces have an atypically high adhesive energy per unit length. This idea suggests that in a nearly normal limb the adhesive energy increases as interfaces deviates in either direction from normal. The adhesive energy will also increase with copies, since the additional interfaces in supernumerary structures contribute to the adhesive energy. Thus the adhesive energy landscape has a local minimum at a point corresponding to a normal limb.

As Fig. 4C shows, the strain energy increases with copies, since extra copies are associated with increased curvature of the limb surface near the supernumerary tips. As with the adhesive energy landscape, the strain energy landscape has a local minimum at the normal value of copies, 1.

At each point in the state plane the total energy is the sum of the adhesive energy and the bending strain energy. From the preceding arguments, the point corresponding to a normal limb is at a local minimum of the total energy. However, this minimum is not accessible to a limb regenerating after the grafting operation in Fig. 1. If the right-handed graft does not degenerate, or does not dedifferentiate and then redifferentiate to become a left-handed tip, then the host-graft combination must become something other than a normal left-handed limb. Evidently the local rules governing the dynamics of regeneration constrain the trajectory of regeneration. These rules, which are not well understood, govern the spatio-temporal patterns of cell movement, division, and differentiation (O'Brochta and Bryant, 1986). Implicitly, the preceding discussion has assumed that cells behave in ways which reduce a local energy density, the sum of densities for adhesive and strain energy.

Without knowing the local rules, we can locate the band within which trajectories of regeneration must lie: The regeneration band does not contain the point representing the normal limb. If the post-operative limb initially has a sub-normal number of interfaces,

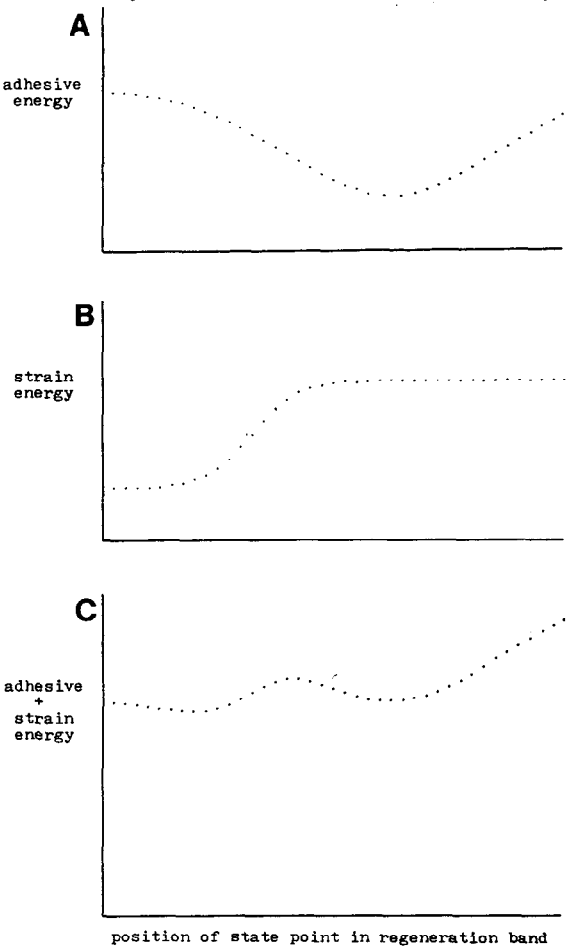

FIG. 5. Sections through the energy landscape, lying within the regeneration band. (A) Adhesive energy (Figs. 4B, 4D), here represented by

$$y = 2 - \exp\{-a(x-b)^2\}$$

with a = .6 and b = 2.5. (B) Strain energy (Figs. 4C, D), here represented by

$$y = 1 + \{1 + \exp[-c(x-d)]\}^{-1}$$

with c = 5 and d = 1.3. (C) Total energy, the sum of (A) and (B), has two minima; some other choices of parameters do not give two minima. This graph represents a section of the energy landscape in Fig. 6, perpendicular to the state plane and intersecting it within the regeneration band.

the principle of continuity implies that this number will increase during regeneration. The principle also implies that copies also increases as regeneration proceeds. Thus, as Fig. 4D shows, the regeneration band lies to the upper right of the point representing the post-operative limb.

The restriction of developmental trajectories to the regeneration band implies that the energy landscape does not determine the whole dynamics of regeneration. However, I suggest that the system develops in a direction "downhill" in energy, to the extent that the local rules allow it. On this hypothesis, stable forms of limbs which can regenerate from an initial post-operative state correspond to local minima of the energy within the regeneration band. To locate these minima, one can place the regeneration band like a window over the topographic map of the adhesive energy landscape, Fig. 4B. Imagine taking a walk along the band through this landscape, starting from the point for the post-operative limb. As Fig. 5A shows, the route starts at a high energy, descends to a lower energy, and rises again to a higher energy. During a similar walk over the strain energy landscape our route rises monotonically (Fig. 5B). These contributions from the adhesive and strain energy energy landscapes add together to define a route on the total energy landscape; the route has two minima separated by a maximum (Fig. 5C).

Thus on the topographic map of the total energy landscape, shown in Fig. 6, a ridge separates two valleys. The right-hand valley represents triplicated limbs, in which the adhesive energy is relatively low (because there are no pattern discontinuities) but the strain energy is relatively high (because the two supernumerary tips are highly curved). The left-hand valley represents duplicated limbs, in which the adhesive energy is relatively high (because a pattern discontinuity occurs), but the strain energy is lower with two tips than with three.

The transition from trip to dup can not occur because the two supernumerary tips of a trip, once differentiated, are stable. However, a dup may regenerate to a trip, increasing the strain energy but lowering the adhesive energy. This asymmetry of transitions would explain the observed prevalence of triplicated limbs.

CONCLUSION

A biomechanical model which treats an epithelium as a fluid elastic shell offers an interpretation for the principle of continuity and for violations of the principle. The model suggests that regeneration should restore the normal pattern of structures if a normal structure has the pattern of cell types and the shape with the lowest energy. Structures with discontinuities of pattern may represent metastable local minima, attained by alternative compromises between strain and adhesion.

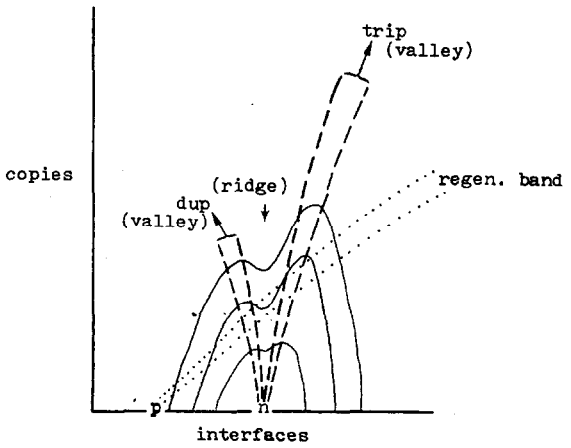

FIG. 6. Landscape for total energy; cf. Fig. 4.

These results may be describable by catastrophe theory, which analyzes discrete alternative states emerging from an underlying continuous dynamic with variation of parameters (Poston and Stewart, 1978). Here a parameter which can be varied to alter the pattern of regeneration is the length of the graft. As the graft length approaches zero, a unique regenerate -- the normal limb -- is attained. However, dup or trip limbs may occur with longer grafts. The shell model suggests that an interpretation in terms of elementary catastrophe theory may be possible, since the shape of the epithelium is postulated to be derivable from an energy functional. Although catastrophe theory has usually been applied to a system with a finite number of degrees of freedom, recently it has been used to analyze a bifurcation in the morphology of a soap film -- a system which is continuous and therefore has an infinite number of degrees of freedom (see Stewart, 1984). Thus the shell model offers mathematical biologists a new opportunity to relate morphogenesis to catastrophe theory.

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REFERENCES

French, V., P. J. Bryant, and S. V. Bryant (1976). Pattern regulation in epimorphic fields. Science 193, 969-981.

Lewis, J. (1981). Simpler rules for epimorphic regeneration: The polar coordinate model without polar co-ordinates. J. Theor. Biol. 88, 371-392.

Mittenthal, J. E. (1981). The rule of normal neighbors: A hypothesis for morphogenetic pattern regulation. Developmental Biology 88, 15-26.

Mittenthal, J. E. and R. M. Mazo (1983). A model for shape generation by strain and cell-cell adhesion in the epithelium of an arthropod leg segment. J. Theor. Biol. 100, 443-483.

Mittenthal, J. E. and R. M. McMeeking (1986). Morphogenesis of epithelia: The shaping of a fluid elastic shell. J. Theor. Biol. (in press).

Mittenthal, J. E. and R. Warga (1983). The shaping of triplicated tips in crayfish legs. In J. F. Fallon and A. I. Caplan (Eds.), Limb Development and Regeneration, Part A. Alan R. Liss, Inc., New York, pp. 619-628.

O'Brochta, D. A. and P. J. Bryant (1986). Patterns of growth during the regeneration of Drosophila imaginal wing discs. Developmental Biology (in press).

Poston, T. and I. N. Stewart (1978). Catastrophe Theory and its Applications, Pitman, London.

Stewart, I. (1984). Soap bubbles and catastrophes. Nature 309, 581.

Winfree, A. T. (1980). The Geometry of Biological Time, Springer-Verlag, New York.